

## DISCRETE CHLOROPHYLL FILTRATION PROCEDURE

### GET IT TOGETHER:

- square 250ml PP & 125ml PP bottles for measuring sample volume
- 0.45um 47mm HAWP filters
- magnetic filtration towers
- forceps
- clean glass scintillation vials with inner-poly lids not inner-foil lids - acetone reacts with foil
- Sharpie

### PREPARE REAGENTS

#### 90% acetone - use volumetric flasks for preparation to ensure volume accuracy

- Measure 100ml of Nanopure into a 1000ml volumetric flask.
- Fill flask to 1000ml volumetric mark with acetone - use a disposable pipette if necessary.
- Cover with stopper or parafilm and invert several times.
- Make sure that volume of liquid is at correct mark on neck of flask, if not, add more acetone using a disposable pipette.
- Pour contents of flask into 1L amber glass bottle (re-use the acetone bottles) marked 90% ACETONE.

#### 10% HCl

- Measure 10ml HCl into a 100ml volumetric flask.
- Fill to 100ml volumetric mark with Nanopure.

### THINGS TO REMEMBER:

- 1) Do not pre-fill glass scintillation vials with 90% acetone, acetone evaporates!
- 2) Calibrate acetone dispenser every time you fill it!!!
- 3) Record sample information on log sheet and assign consecutive numbers to samples/blanks prior to processing. Using consecutive numbers makes labelling the vials easier.
- 4) Be sure everyone is following same procedure.

### SAMPLE COLLECTION:

- Use a square 250ml PP or 125ml PP bottle to measure sample.
- Rinse bottle and cap 3 times with water from the carboy.
- Fill bottle to brim and cap. Filter samples immediately.

### SAMPLE FILTERING:

#### Blank Preparation:

- Place a blank filter (HAWP 0.45um) in a glass scintillation vial. Label the lid with the date and "BLANK". Do a blank for every box (100) of filters.

#### Sample Preparation:

- Place filter on base with tweezers; do not use fingers, seat magnetic funnel, do not crease filter.
- Shake sample container, then pour seawater sample into filter funnel.
- Turn vacuum pump on; vacuum should be around 10 mm Hg. Pump only until filter dries, do not maintain vacuum on dry filters.
- Carefully fold filter with forceps and place in glass scintillation vial (fold filter in thirds and it will slide into vial easily), cap tightly.
- Label cap of scintillation vial as per labeling protocol. Keep covered with aluminum foil until all samples have been filtered. If samples are not to be extracted immediately, place tray in freezer and keep filters frozen until they can be processed (up to 3 months).
- Add 10ml of 90% acetone to all vials (don't forget to calibrate dispenser!), CAP TIGHTLY, shake to dissolve filter. Cover with foil and place in freezer, KEEP UPRIGHT, samples will leak if tipped over.
- Extract in the freezer for 24-48 hours. Note time on log.
- Shake the samples at least once during this period. Do not shake 4 hours prior to reading – particulate matter will be re-suspended in sample volume.

### LOG EVERYTHING:

Record ALL information to log sheet. Make a note on the log sheet if any problems were encountered during filtration; i.e. a torn filter, leaking funnel - ANYTHING. Fill out entire log sheet – it may take a little time now, but it's worth it in the long run! Check your labeling.